

1 Reliability of serological tests for COVID-19: Comparison of three immunochromatography
2 test kits for SARS-CoV-2 antibodies.

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25 **Abstract**

26 **Background:** Several immunochromatographic serological test kits have been developed to detect
 27 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antibodies, but their
 28 relative performance and potential clinical utility is unclear.

29 **Methods:** Three commercially available serological test kits were evaluated using 99 serum samples
 30 collected from 29 patients diagnosed with coronavirus disease 2019 (COVID-19).

31 **Results:** The IgM antibody-positive rates of the three serological test kits for samples taken at the
 32 early stage of the disease (0–6 days after onset) were 19.0%, 23.8%, and 19.0%, respectively. The
 33 IgM antibody-positive rates over the entire period were 21.2%, 60.6%, and 15.2%, respectively. The
 34 IgG antibody-positive rates for samples taken after 13 days of onset were 100.0%, 97.6%, and 97.6%,
 35 respectively.

36 **Conclusion:** There were large differences among the results of the three test kits. Only few cases
 37 showed positive results for IgM in the early stage of disease and the IgM antibody-positive rates
 38 over the entire period were low, suggesting that the kits used in this study were unsuitable for
 39 diagnosis of COVID-19. The IgG antibody was positive in almost all samples after 13 days of onset,
 40 suggesting that it may be useful for determining infections in the recent past.

41

42 **Keywords**

43 SARS-CoV-2; COVID-19; antibody; immunochromatography

44

45 **1. Introduction**

46 Coronavirus disease 2019 (COVID-19) has been spreading globally. COVID-19 is caused
47 by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which was confirmed
48 around December 2019 in Wuhan, Hubei, China [1, 2]. Currently, real-time reverse
49 transcription-polymerase chain reaction (RT-PCR) is employed to detect SARS-CoV-2 in a
50 nasopharyngeal swab or sputum for the diagnosis of COVID-19 [3]. However, RT-PCR gives
51 false-negative results in cases of a low viral titer and inadequate sample collection. Studies in China
52 using RT-PCR tests reported that only 60%–70% of COVID-19 patients were positive in the early
53 stages of infection [3-6].

54 Detection of specific antibodies to a pathogen in the bloodstream is widely used to
55 diagnose infectious diseases. An antibody test using a blood sample is relatively quick and
56 straightforward, and because the risk of infection during the sample collection process is low, it is
57 considered by some to be useful for the diagnosis of COVID-19 [7]. Generally, antigen-specific IgM
58 antibodies increase in the early stage of the onset of a viral infection, which is then followed by an
59 increased level of specific IgG antibodies. IgM antibodies are produced as the first antibody to fight
60 the virus and are transiently raised. The subsequent production of IgG antibodies continues to rise
61 for a long time and plays a vital role in immunity against the same virus. Therefore, the detection of
62 specific IgM antibodies against SARS-CoV-2 may be used for diagnosis in the acute phase of

COVID-19, whereas the detection of SARS-CoV-2-specific IgG antibodies may be used for determining a past infection or acquired immunity against SARS-CoV-2.

Immunochromatographic anti-SARS-CoV-2 antibody detection kits have been recently developed by multiple manufacturers [8]. However, differences in their properties and clinical usefulness are mostly unknown [9]. This study aimed to investigate the reliability of three different immunochromatographic anti-SARS-CoV-2 antibody detection kits using serum from COVID-19 patients.

70

71 2. Materials and Methods

72 2.1. Detection of anti-SARS-CoV-2 antibodies

We evaluated three test kits for the detection of anti-SARS-CoV-2 IgM/IgG antibody in serum: 2019-nCoV IgG/IgM Rapid Test Cassette (Hangzhou AllTest Biotech Co., Ltd., China), COVID-19 IgM/IgG Duo (SD BIOSENSOR, Korea), and 2019-nCoV IgG/IgM Detection Kit (Vazyme Biotech Co., Ltd., China). All kits were based on colloidal gold-labeled immunochromatography. All tests were performed following the manufacturer's instructions for each kit, and the results were visually inspected within 15 min.

79

80 2.2. Study subjects

81 We utilized a series of residual serum samples left over after routine laboratory testing of
82 29 COVID-19 patients (mean age, 52.9 ± 21.9 years; 14 males and 15 females) who were admitted
83 to Fujita Health University Hospital, Toyoake, Japan, from February 28 to April 15, 2020. All
84 patients were confirmed as COVID-19 cases by RT-PCR assay of nasopharyngeal swab specimens
85 at the time of or prior to admission. All serum samples, aliquoted and stored at -80°C , were thawed
86 and evaluated at the same time for the analyses. This study was approved by the Ethics Committee
87 for Clinical Research, Center for Research Promotion and Support, Fujita Health University
88 (authorization number HM19-493).

89

90 3. Results

91 3.1. Positive rates of anti-SARS-CoV-2 IgM antibody

92 Table 1 shows the results of anti-SARS-CoV-2 IgM antibody in patients' serum according
93 to the number of days after disease onset. The IgM antibody-positive rate of the three kits in the
94 early stage (0–6 days after onset) was 19.0% (Hangzhou AllTest), 23.8% (SD BIOSENSOR), and
95 19.0% (Vazyme Biotech), showing comparable results among the kits. On the other hand, the IgM
96 antibody-positive rate 7 days after the onset showed considerable differences among the kits. After
97 13 days of onset, the IgM antibody-positive rates were 23.8%, 88.1%, and 14.3% for the Hangzhou
98 AllTest, SD BIOSENSOR, and Vazyme kits, respectively. The SD BIOSENSOR kit showed a

99 particularly high IgM antibody-positive rate (Table 1). The total IgM antibody-positive rates over the
100 entire period were 21.2%, 60.6%, and 15.2% for the Hangzhou AllTest, SD BIOSENSOR, and
101 Vazyme kits, respectively.

102

103 3.2. Positive rate of anti-SARS-CoV-2 IgG antibody

104 Table 2 shows the results of anti-SARS-CoV-2 IgG antibody in patients' serum according
105 to the number of days after onset. The IgG antibody-positive rates in the early stage (0–6 days after
106 onset) were 33.3%, 33.3%, and 28.6% for the Hangzhou AllTest, SD BIOSENSOR, and Vazyme
107 kits, respectively. Patients positive for IgG antibody were observed at the early stage of the onset.
108 The IgG antibody-positive rates after 13 days of the onset were 100%, 97.6%, and 97.6%,
109 respectively, showing that the three kits gave positive results for almost all samples. Note that, since
110 the IgM antibody-positive samples were also IgG antibody-positive, the positive rate of either or
111 both IgM and IgG antibody was the same as the positive rate of the IgG antibody (Table 2).

112

113 3.3 Kinetics of anti-SARS-CoV-2 IgM and IgG antibody

114 Figures 1 and 2 show the kinetic results of anti-SARS-CoV-2 IgM and IgG antibody of the
115 29 patients in our cohort. Few cases showed positive test results for IgM early in the disease course.
116 Tests for the IgG antibody often turned positive about 10 days after the onset, and most of the

117 samples after 13 days of the onset became positive with all kits. However, there was a dissociation
118 of the results in patient 11, where the AllTest kit gave positive results in all samples, whereas the SD
119 BIOSENSOR and Vazyme kits gave negative results.

120

121 **4. Discussion**

122 The serological detection of antibodies is widely used for the diagnosis of viral infections.
123 Since immunochromatography methods to detect antibodies are quick to perform, easy to use, and
124 do not require additional equipment, detection of anti-SARS-CoV-2 antibody using
125 immunochromatography kits may be helpful for diagnosing COVID-19.

126 We compared three immunochromatography kits for detecting anti-SARS-CoV-2
127 antibodies using serum samples from COVID-19 patients. There was a considerable difference
128 among the kit results, especially for IgM. It is thus clear that kit selection is crucial and should be
129 based on the clinical purpose for the test. Additionally, since only a few samples were positive for
130 the IgM antibody in the early stage of the disease by all kits, it appears that the kits used in this study
131 are unsuitable for diagnosing the acute phase of COVID-19.

132 A comparison of the IgG antibody results between the three kits did not reveal any large
133 differences. Additionally, almost all serum samples collected after 13 days of onset were positive.
134 This suggests that the IgG antibody test using these kits can be helpful in diagnosing COVID-19

135 after a certain period from disease onset. However, the results of one patient were positive for one
136 kit and negative for the other two kits, suggesting the presence of false-positive and false-negative
137 results. Since research regarding the cause of false-positive and false-negative results in
138 anti-SARS-CoV-2 antibody tests has yet to be performed, further investigation is necessary, such as
139 determining the presence of cross-reactions with other coronaviruses. Furthermore, it is unclear how
140 long anti-SARS-CoV-2 IgG antibody levels continue to rise in serum after infection, and further
141 research is warranted.

142 Currently, epidemiological studies in several countries use immunochromatography kits to
143 detect antibodies [10]. We suggest that when using immunochromatography kits for COVID-19
144 diagnosis, particular attention must be paid to whether these kits detect neutralizing antibodies
145 against SARS-CoV-2. SARS-CoV-2 contains at least four structural proteins (spike [S] protein,
146 envelope protein, membrane protein, and nucleocapsid [N] protein) [11, 12]. Since S protein appears
147 to be involved in adhesion between virus and host cells during infection, it is considered that an
148 antibody against S protein acts as a neutralizing antibody [13-15]. The detection of antibodies by
149 immunochromatography kits may be useful for determining a previous virus infection, but it is
150 unclear whether the results could indicate people who have gained acquired immunity. Besides,
151 quantitative assay of anti-SARS-CoV-2 antibodies, such as ELISA, would give a better
152 understanding of humoral immune response to COVID-19 infection over time.

153

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156 2019-nCoV IgG/IgM Rapid Test Cassette and COVID-19 IgM/IgG Duo kits and the 2019-nCoV

157 IgG/IgM Detection Kit, respectively.

158

159 **Declaration of interest**

160 Hidetsugu Fujigaki received immunochromatographic anti-SARS-CoV-2 antibody detection kits

161 from Nichirei Biosciences Inc. and Shionogi & Co., Ltd. There are no other relationships or

162 activities that could appear to have influenced this work. All other authors report no conflicts of

163 interest relevant to this work.

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209

210 **Figure legends**

211

212 **Figure 1. Kinetics of anti-SARS-CoV-2 IgM antibody of serum samples from 29 COVID-19**

213 **patients.** The positive or negative result of the antibody in each serum sample is expressed as + or −,

214 respectively. A, Hangzhou AllTest; B, SD BIOSENSOR; C, Vazyme.

215

216 **Figure 2. Kinetics of anti-SARS-CoV-2 IgG antibody of serum samples from 29 COVID-19**

217 **patients.** The positive or negative result of the antibody in each serum sample is expressed as + or −,

218 respectively. A, Hangzhou AllTest; B, SD BIOSENSOR; C, Vazyme.

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220

Days after onset	No. of samples	IgM antibody					
		Hangzhou AllTest		SD BIOSENSOR		Vazyme	
		No. of positive samples	Positive rate (%)	No. of positive samples	Positive rate (%)	No. of positive samples	Positive rate (%)
0-6	21	4	19.0	5	23.8	4	19.0
7-8	10	1	10.0	2	20.0	0	0.0
9-12	26	6	23.1	16	61.5	5	19.2
After 13	42	10	23.8	37	88.1	6	14.3
Total	99	21	21.2	60	60.6	15	15.2

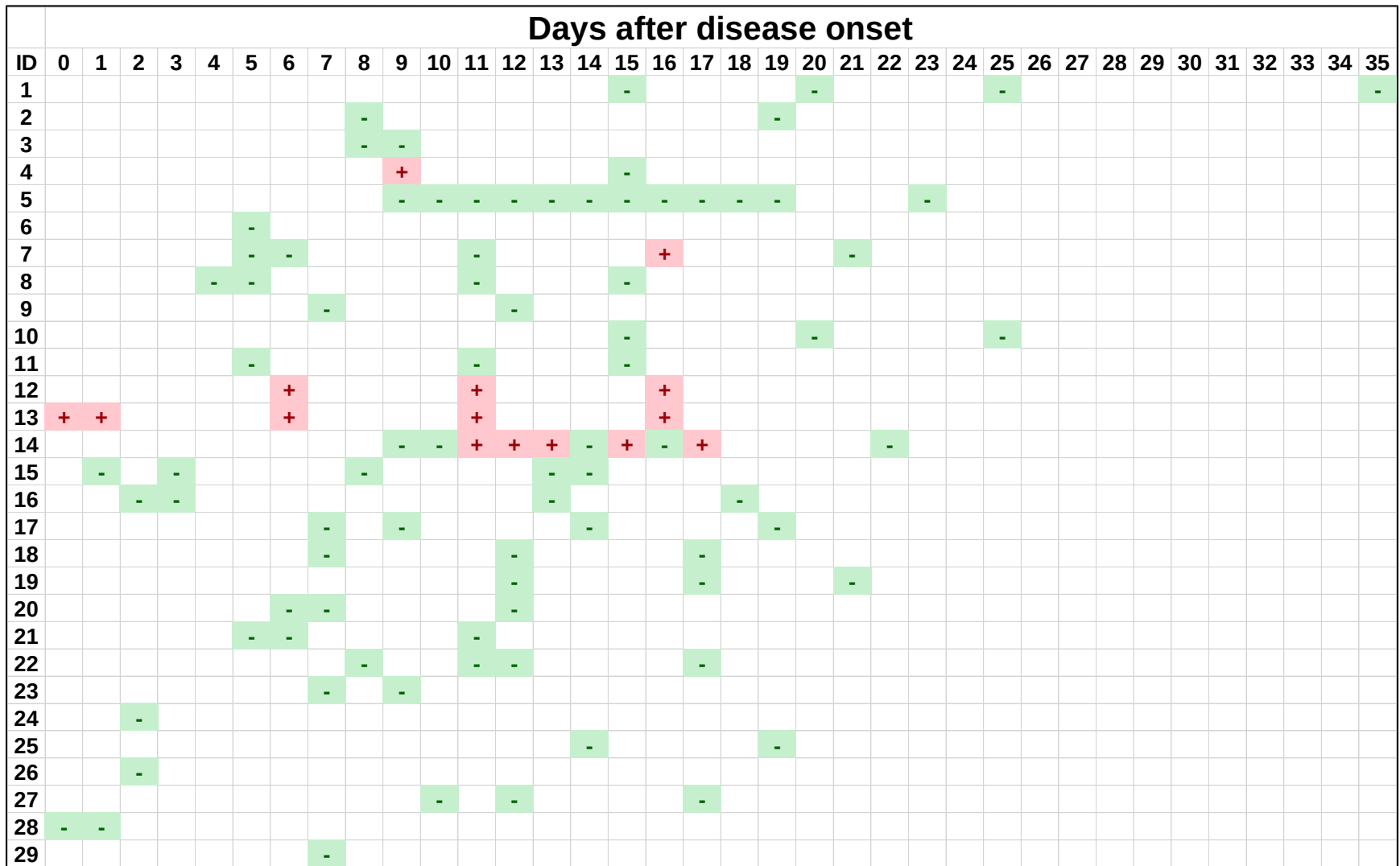
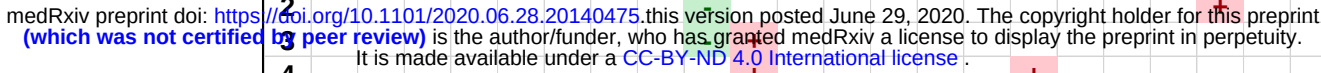
Table 1. Positive rate of anti-SARS-CoV-2 IgM antibody according to the different kits used

223 **Table 2. Positive rate of anti-SARS-CoV-2 IgG antibody according to the different kits used**

Days after onset	No. of samples	IgG antibody					
		Hangzhou AllTest		SD BIOSENSOR		Vazyme	
		No. of positive samples	Positive rate (%)	No. of positive samples	Positive rate (%)	No. of positive samples	Positive rate (%)
0–6	21	7	33.3	7	33.3	6	28.6
7–8	10	3	30.0	3	30.0	3	30.0
9–12	26	19	73.1	18	69.2	17	65.4
After 13	42	42	100.0	41	97.6	41	97.6
Total	99	71	71.7	69	69.7	67	67.7

224 The positive rate of either or both IgM and IgG antibody was the same as the positive rate of the IgG
225 antibody.

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A



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